International Journal of Agricultural Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 7, Issue 4, Aug 2017, 651-656

GENE EXPRESSION ANALYSIS IN ROOTS OF RICE (*ORYZA SATIVA* L.) UNDER VARIED ZINC CONDITION

HEMAVATHI[1], BRIJESH A. S[2] & SHASHIDHAR H. E[3]

[1,3]*Department of Plant Biotechnology, University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India*

[2]*Department of Crop Improvement & Biotechnology, College of Horticulture, UHS Campus, GKVK, Karnataka, India*

ABSTRACT

Zinc (Zn), is an important element essential to both plant and humans which play key role in gene expression, cell development and replication processes. Zinc deficiency is prevalent and widespread in rice consuming countries which causes delayed plant development and decreased crop yield. Many studies have investigated the response of rice plants to Zn deficiency using high and low zinc accumulating genotypes at physiological and biochemical level. However, little information is available about mechanisms at molecular level. Therefore, an attempt was made to understand the Zn deficiency in the roots of rice seedlings through transcriptome analysis. The results revealed that a diverse set of differentially expressed genes (DEGs) with distinct functions were altered under zinc deficient condition as compared to zinc supplemented condition. DEGs were majorly involved in primary metabolism such as carbohydrate, lipid, protein and secondary metabolism. KEGG pathway analysis revealed that majority of DEGs were upregulated and down regulated in biosynthesis of terpenoids and steroids, oxidative phosphorylation, nitrogen metabolism, citrate cycle pathways. The present result reveal that zinc deficiency leads to activation of several genes and gene networks, indicating how plants cope with the micronutrient deficiency at different developmental stages of rice.

KEYWORDS: Expression Analysis, Differentially Rice Root, Transcriptome & Zinc Deficient Expressed Genes

Received: Ju1 02, 2017; **Accepted:** Jul 28, 2017; **Published:** Aug 05, 2017; **Paper Id.:** IJASRAUG201784

Original Article

INTRODUCTION

Zinc (Zn) is an essential trace element required for all the living organisms. It is a main co-factor required by many enzymes and proteins (Broadley *et al*, 2007). Zn deficiency is the most prevalent micronutrient disorder in the rice growing regions (Sillanpaa, 1990). The noticeable symptoms of Zn deficiency in rice are leaf bronzing, decreased growth and delayed development resulting in reduced grain yield (Widodo *et al*, 2010). Zn deficiency can be alleviated by the through application of Zn fertilizers and conventional plant breeding. High Zn accumulating varieties developed through plant breeding are capable of growing better on soils with low available Zn, compared with low Zn varieties. Developing high Zn varieties causes efficient Zn utilization (Suzuki *et al.*, 2008), through higher Zn uptake by roots which is achieved by the exudation of phytosiderophores, such as deoxymugineic acid, or low-molecular-mass organic acids, such as malate and citrate (Duffner *et al*, 2012).Zn deficiency induces expression of various Zn transporters and metal chelators (Durmaz*et al*, 2011) at transcriptional level. To our best knowledge, two studies have analysed the transcriptome changes under Zn deficiency in different rice varieties using conventional microarray and explored the genes involved in Zn homeostasis (Suzuki et al. 2012).Therefore, we investigated transcriptome profiles of high Zn accumulating recombinant inbreed line (RIL) under Zn supplied and deficient condition and identify genes and biological pathways involved in the Zn supplied

and deficient condition.

MATERIALS AND METHODS

Experiment was conducted with high zinc RIL grown under Zn deficient and supplemental conditions. For micro array study, the samples were collected as follows; rice seeds of high zinc RIL AM72 was germinated on paper soaked with distilled water. After germination, the seedlings were transferred to a thermocol floater on medium containing nutrient solution and distilled water (DW) in greenhouse. The composition of the nutrient solution was as follows: 0.70 mM K2SO4, 0.10 mMKCl, 0.10 mM KH2PO4, 2.0 mMCa (NO3)2, 0.50 mM MgSO4, 10 µM H3BO3, 0.50 µM MnSO4, 1.00 µM ZnSO4, 0.20 µM CuSO4, 0.01 µM (NH4)6Mo7O24, and 36 µMFeNaEDTA. The pH of the nutrient solution was adjusted daily to 5.5 with 1 M HCl, and the nutrient solution was renewed every 4 days. For the Zn deficiency and supplemented treatments, one-week-old rice plants were transferred to nutrient solution containing 0.05 µM (Zn deficiency), or 1.0 (Zn supplement) µM ZnSO4 and grown for three weeks. The roots from four week old plants were collected and pooled into two biological replicates. The pooled roots were freeze dried in liquid nitrogen and further used for RNA extraction. Total RNA was isolated using Trizol Reagent according to manufacturer's protocol and RNA was quantified using nano drop spectrometer (Agilent). For microarray analysis, RNA was labeled using an Agilent Low RNA Input Linear Amplification Kit (Agilent Technologies., India), following the manufacturer's instructions. The microarray analyses were performed with the two biological replicates for each treatment. The list of genes present in significant functions and pathways are obtained using DAVID database (http://david.abcc.ncifcrf.gov/home.jsp).

RESULTS AND DISCUSSIONS

In the present study, hydroponically grown high zinc RIL AM72 rice seedlings (1-weeks old) were transferred to Zn free solution (Zn−) and Zn supplemented solution (Zn+, 1 µM) as treatment, respectively. There was no phenotypic difference between Zn− and Zn+ seedlings for one week. The seedlings showed obvious phenotypic differences after 7 days, at which time the Zn− plants had smaller roots and shoots and leaves began to decolorize. Further, the rice seedlings grown under Zn− condition showed reduced root and shoot growth than those of the Zn+ seedlings (Figure 1). The rice seedlings grown in distilled water as controlled condition completely decolorized with severe stunted growth by the end of fourth week. Analysis of the growth of rice under Zn deprived (4 week) revealed chlorosis (bleaching) of older leaves, as well as a reduction in the growth of seedlings as compared to Zn supplemented condition (Bandyopadhyay *et al*, 2017).

DW Zn- Zn+

Figure 1: Phenotypes of Rice Seedlings under Control, Zn Supplement and Zn Deficient Conditions

To evaluate the potential functions of the differentially expressed genes (DEGs) in response to Zn deficiency, gene ontology (GO) analysis was performed. A large number of DEGs were found to be involved in metabolic processes, particularly primary metabolism (carbohydrate, lipid, protein and nucleic acid) and secondary metabolism. Zinc is a crucial ion for plants as it acts as a cofactor for various enzymes in different metabolic pathways (Ishimaru*et al*, 2011). From this gene expression analysis, many genes related to metabolism show edaltered expression in Zn deficient condition as compared to Zn supplemented condition. Among carbohydrate metabolism category genes such as phosphoenol pyruvate kinase (Os12g0189300; 2.49), UDP-glucosepyrophosphorylase (Os02g0117700; 1.71) were upregulated and phosphoglucomutase precursor (Os06g0476200; 4.22), starchsynthase (Os06g0160700; 1.22) were down regulated. Present microarray data suggests a number of proteins involved in diverse aspects of carbohydrate metabolism were differentially regulated and thereby indicates that zinc deficiency has widespread effects on carbohydrate metabolism. Carbohydrate metabolism has direct impact on plant biomass and yield indicating that the up regulation of the carbohydrate metabolism genes could be due to the stress caused by zinc deprivation. Previously, it has been reported in *Arabidopsis* involvement of lipases and their similar differential expression was due to zinc deficiency treatments (Suzuki et al. 2012). Under lipid metabolism category, Omega-6 fatty acid desaturates (Os07g0416900; 2.52), UDP-glucuronosyl/UDP-glucosyl transferase family gene (Os09g0518200; 1.13) were unregulated and indole-3-acetate-β-glucosyl transferase gene (Os03g0693600; 1.31), phospholipase D (Os06g0604200; 1.26) were down regulated. In Zn deficient conditions, the majority of DEGs not only belonged to carbohydrate and lipid metabolism, but several genes were also involved with protein and nucleic acid metabolism. Protein metabolism related genes such as serine carboxy peptidase family (Os05g0582800; 1.83) aspartic proteinase (Os11g0184600; 1.15) and nucleic acid metabolism gene CCT motif family protein (Os11g0101200; 0.71) were upregulated (Table 1)

Table 1: Classification of Differentially Expressed Genes in Various Categories in Zinc Deficient Condition as Compared to Zinc Supplemented Condition

Gene ID	Gene description	Fold level	Zn-/Zn+
Carbohydrate metabolism			
Os03g016800	Lactose permease, sugar transport protein	2.56	up
Os12g0189300	Pyruvate/Phosphoenol pyruvate kinase	2.49	up
Os01g0276700	Pyruvate kinase	1.76	up
Os02g0117700	UDP-glucose pyrophosphorylase	1.71	up
Os05g0194900	Pyrophosphate-dependentphosphofructo-1-kinase-like protein	1.53	up
Os12g0514000	Sorbitol transporter, Sugar transporter family protein, expressed	1.17	up
Os07g0530600	pyruvate, phosphate -di-kinase regulatory protein	1.16	up
Os06g0476200	Phosphoglucomutase precursor	4.22	down
Os06g0160700	Starch synthase	1.22	down
Os03g0267300	Fructose-1,6-bisphosphatase	1.15	down
Os09g0298200	Glucose-1-phosphate adenylyl transferase	0.94	down
Os03g0401300	Sucrose synthase	0.74	down
Lipid metabolism			
Os07g0416900	Omega-6 fatty acid desaturase	2.52	up
Os09g0518200	UDP-glucuronosyl/UDP-glucosyl transferase family protein	2.13	up
Os03g0289800	Oxidoreductase, 2OG-Fe oxygenase family protein	1.59	up
Os01g0858350	cytochrome P450-dependent fatty acid hydroxylase	1.27	up
Os06g0226950	Acid phosphatase	1.50	down
Os03g0693600	Indole-3-acetate-β-glucosyl transferase	1.31	down
Os06g0604200	Phospholipase D	1.26	down
Os05g0468500	Esterase, SGN Hhydrolase-type domain containing protein	1.22	down
Os12g0628400	Fatty acid hydroxylase	1.04	down
Protein metabolism			
Os05g0582800	Peptidase S10, serine carboxy peptidase family protein	1.83	up
Os11g0184600	Aspartic proteinase (Asp1)	1.15	up
Os12g0257000	Serine carboxy peptidase-I precursor	0.73	up
Nucleic acid metabolism			
Os11g0101200	CCT motif family protein	0.74	up

The secondary metabolism category included many enzymes such as homocysteine S-methyl transferase 1(Os03g0221200; 1.94), terpene synthase (Os03g0428200; 1.65), aldo/ ketoreductase family (Os04g0339400; 1.40), oxidoreductase, 2OG-Feoxygenase family protein (Os10g0559200; 1.03) were upregulated. Similarly, enzymes such as GAC 20oxidase2 (Os01g0883800; 2.03), cytochrome P450 (Os09g0403300; 1.87), mitochondrial aldehyde dehydrogenase (Os02g0730000; 1.57), O-methyl transferase family protein (Os11g0305400; 1.14) were downregulated (Table 2).These results indicate that Zn deficiency induces production of metabolites involved in secondary metabolism. According to previous reports, genes involved in secondary metabolism were mainly linked to zinc deficiency (Bandyopadhyay *et al*, 2017 and Nanda *et al*, 2017) in rice roots.

Table 2: Differentially Expressed Genes in Secondary Metabolism Category in Zinc Deficient Condition as Compared to Zinc Supplemented Condition.

Gene ID	Gene description	Fold level	Zn-/Zn+
Secondary metabolism			
Os01g0209700	GA 2-oxidase 5	2.15	up
Os04g0179700	Syn-pimara-7,15-diene synthase	2.06	up
Os03g0221200	Homocysteine S-methyl transferase 1	1.94	up
Os03g0428200	Terpene synthase, N-terminal domain containing protein	1.65	up
Os04g0339400	Aldo/keto reductase family protein	1.40	up
Os10g0559200	Oxidoreductase, 2OG-Feoxygenase family protein	1.03	up
Os10g0516300	Flavoprotein-ubiquinone oxidoreductase	0.79	up
Os04g0665200	Indole-3-acetate O-methyl transferase 1	2.49	down
Os01g0883800	GAC 20oxidase2	2.03	down
Os09g0403300	cytochrome P450	1.87	down
Os02g0730000	Mitochondrial aldehyde dehydrogenase	1.57	down
Os03g0699700	Linoleate 9S-lipoxygenase 1	1.47	down
Os11g0305400	O-methyl transferase family protein	1.14	down

Pathway-based analysis was used to further understand the biological functions of the DEGs. The biological pathways of the DEGs were identified using KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis. Most significantly enriched pathway identified were biosynthesis of terpenoids and steroids, oxidative phosphorylation, biosynthesis of alkaloids, linoleic acid metabolism, nitrogen metabolism, fructose and mannose metabolism, citrate cycle (TCA cycle) (Figure 2). Alteration in genes involved in TCA cycle suggests that they may play an important adaptive role under Zn deficiency in rice. The balance of energy metabolism between assimilation and dissimilation process is very important for the maintenance of cell life (Huner *et al*, 1998). Most of the DEGs involved in the nitrogen metabolism and oxidative phosphorylation were unregulated indicating their role in the maintenance of cell under Zn deficient condition.

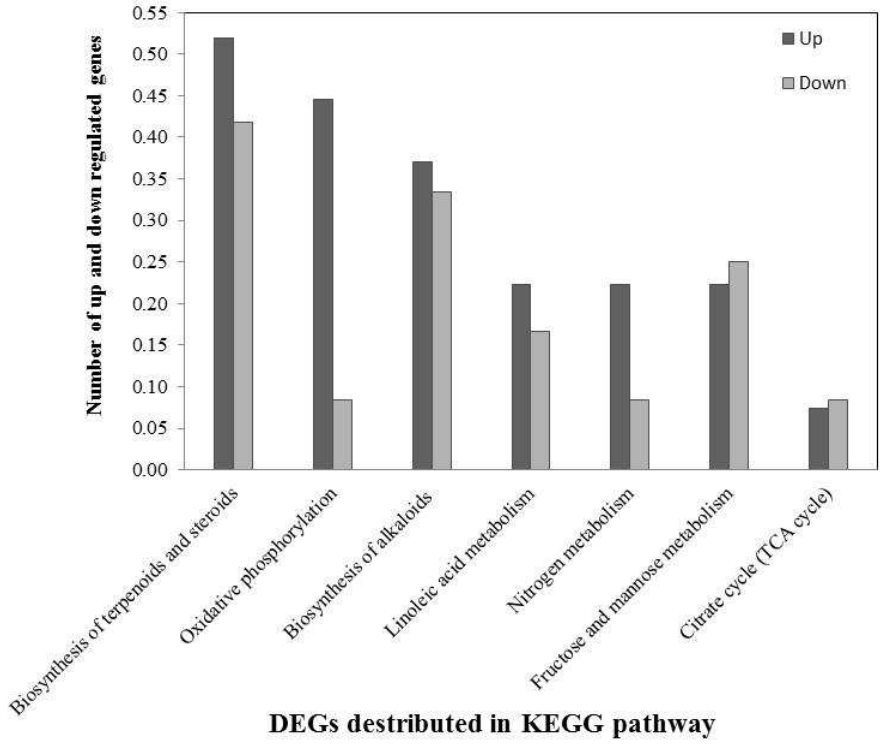

Figure 2: KEGG Pathway Assignment for Differentially Expressed Genes

CONCLUSIONS

Zinc deficiency in plants inhibits plant growth and reduces crop production. In the present study, transcriptomic profiles high zinc accumulating RIL (AM72) rice roots under zinc deficient and supplement condition using Agilent microarray. Zn deficiency showed severe morphological changes. The microarray data showed that several genes were both upregulated and down regulated in response to Zn deficiency as compared to Zn supplementation. The genes mainly upregulated under Zn-condition belong to primary and secondary metabolism indicating their role in Zn metastasis. The present study would allow further investigations of the genes identified and their functions at pathways in improving Zn deficiency tolerance in rice.

ACKNOWLEDGEMENTS

The author gratefully acknowledge for the fellowship and funds received from the DST (WOS-A) grant. Authors also extend their thanks to University of Agricultural Sciences for providing facility to carry out the present work.

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